

US EPA ARCHIVE DOCUMENT

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| PAGE 1 of 29 | METHOD No. AG-349 | SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN ANIMAL TISSUES, MILK AND EGGS AS 2,6- DIMETHYLANILINE |
| EDITION 11 / 25 / 80 | | |
| SUBMITTED BY: K. Balasubramanian | | |

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1.0 SCOPE

This total residue method is used for the determination of the combined residues of metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester] and its metabolites which contain the 2,6-dimethylaniline moiety in animal tissues, milk and eggs. (See Figure 1 for structures). This method is a modification of Analytical Method AG-330 developed originally for tobacco. The limit of detection for the method is 0.01 ppm for milk, 0.05 ppm for muscle and fat tissues and eggs and 0.10 ppm for liver and kidney samples (expressed in metalaxyl equivalents).

2.0 PRINCIPLE

Residues of metalaxyl and its metabolites are extracted from milk by shaking with acetonitrile in a mechanical shaker for 10 minutes. Tissue samples are extracted by blending with 20% water/acetonitrile for 10 minutes. Egg samples are extracted by blending with acetonitrile. The fat samples are extracted by blending with hexane. An aliquot of the extract is partitioned between acetonitrile and hexane to remove the oils and fats which cause interferences in the method. The sample extract is then evaporated and refluxed with phosphoric acid overnight in the presence of cobalt chloride.

The solution is basified and the 2,6-dimethylaniline formed is steam distilled using a modification of the steam distillation apparatus of Veith and Kiwus (2). The steam distilled product is derivatized with trichloroacetyl chloride to minimize problems of volatility of 2,6-dimethylaniline. The derivative is cleaned up by alumina column chromatography and analyzed by gas chromatography using an alkali flame ionization detector (AFID) operating in the nitrogen-specific mode. Liver and kidney samples are subjected to an additional silica gel column cleanup before the gas chromatographic analysis.

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Milk and other samples which have interferences in the AFID detector are analyzed by gas chromatography -mass spectrometry (GLC-MS) in the chemical ionization mode using the M+1 ion at m/e 268. The flow diagram for the method is shown in Figure 1.

3.0 APPARATUS

3.1 Extraction and Filtration

- Food chopper, Hobart or equivalent.
- Blender, Waring or equivalent.
- Variable transformer, Powerstat.
- Funnel, 12.5-cm size.
- Filter paper, Whatman 2V, 32-cm.
- Bottle, Boston Round, narrow mouth, 16-oz.
- Mechanical shaker.
- Round bottom flask, 500-ml.
- Condenser, Allihn bulb-type, 500-mm, water cooled.
- Glascol heating mantle, 500-ml.
- Variable transformer, Powerstat.
- Bottle caps, Poly-Seal lined, 28-mm.

3.2 Partition

- Separatory funnel, 250-ml.
- Round bottom flask, 500-ml.

3.3 Phosphoric Acid Reflux

- Rotary evaporator, Büchi or equivalent.
- Flask, round bottom, 500-ml.
- Condenser, Allihn bulb-type, 500-mm; water cooled.
- Glascol heating mantle, 500-ml.
- Variable transformer, Powerstat.
- Funnel, 8.5-cm size.
- Thermometer 0-360°C, 2-in. immersion.

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3.4 Steam Distillation

- Glascol heating mantle, 500-ml.
- Variable transformer, Powerstat.
- Modified steam distillation apparatus.
(See Figure 3 for details).

3.5 Derivatization

- Separatory funnel, 125-ml.
- Automatic pipette, 100- μ l, Fisher or equivalent.
- Flask, round bottom, 100-ml.
- Funnel, 6.5-cm size.

3.6 Cleanup

- Column, chromatographic, 19-mm i.d., with Teflon stopcock.
- Flask, round bottom 250-ml.
- Graduated concentration tube 20 ml with 24/25 standard taper joint (Kontes K-570050).

NOTE: All the glassware used after the steam distillation step should be rinsed with reagent grade acetone before use to avoid background interference in the GC-AFID detector.

4.0 REAGENTS

- Acetonitrile, distilled in glass, Burdick and Jackson.
- Alumina, basic, (Woelm) W200: Activity Grade V Basic (prepared by addition of 76 ml of water to 324 g of activity grade Super I alumina).
- Phosphoric acid, 85%.
- Cobalt chloride hexahydrate, reagent grade.
- Methanol, reagent grade.
- Hexane, distilled in glass, Burdick and Jackson.
- 25% Sodium hydroxide solution [mixture of equal amounts of distilled water and 50% sodium hydroxide solution (Fisher)].
- Trichloroacetyl chloride, 97%, Aldrich Chemical Company. Catalogue No. 15159-9.
- Dichloromethane, distilled in glass, Burdick and Jackson.
- 5% Aqueous sodium bicarbonate solution.
- Standard metalaxyl.
- Standard N-(2,6-dimethylphenyl)-2,2,2-trichloroacetamide (TCA-DMA).
- Absorbent cotton.
- Silica gel, Davison Chemical Company, Grade H, 100-200 mesh.

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5.0 PROCEDURE

5.1 Sample Preparation

Solid tissues are blended with dry ice (dry ice then allowed to evaporate) or chopped into small pieces. Whole milk samples are thawed (if frozen) and shaken thoroughly to give a uniform mixture. No preparation is required for egg samples.

5.2 Extraction

5.2.1 Extraction of Milk Samples

- 5.2.1.1 Weigh a 50-g representative subsample of milk into a 16-oz. Boston round narrow mouth bottle. Add 250 ml of acetonitrile and cap with a Polyseal lined bottle cap.
- 5.2.1.2 Shake for 10 minutes using a mechanical shaker.
- 5.2.1.3 Filter through a Whatman 2V filter paper into a 16-oz. bottle.
- 5.2.1.4 Transfer a 10-g aliquot (60 ml) of the extract to a 250-ml separatory funnel. Partition with hexane, Step 5.3.

5.2.2 Extraction of of Fat Samples

- 5.2.2.1 Weigh a 15-g representative subsample of fat into a beaker. Transfer the sample to a blender jar using 300 ml of hexane.
- 5.2.2.2 Fit the cap with a polyethylene liner.
- 5.2.2.3 Blend vigorously for approximately 10 minutes to dissolve the fat.

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5.2.2.4 After blending, filter the sample through a Whatman 2V filter paper, collecting the filtrate in a screw cap bottle. NOTE: A small amount of membrane associated with fat tissue will not dissolve in hexane and is filtered out.

5.2.2.5 Transfer a 100-ml aliquot (5-g equivalent) of the sample to a 250-ml separatory funnel. Partition twice with 100 ml of acetonitrile. Combine the acetonitrile fractions in a 500-ml round bottom flask. Discard the hexane phase.

5.2.2.6 Evaporate the sample to dryness using a rotary evaporator (bath temperature 40°C). Proceed to phosphoric acid reflux, Step 5.4.

5.2.3 Extraction of Egg Samples

5.2.3.1 Weigh a 15-g representative sample into a Waring blender. Add 150 ml of acetonitrile.

5.2.3.2 Fit the cap with a polyethylene liner to prevent extraction of extraneous material from the top and to avoid loss of solvent.

5.2.3.3 Blend the sample for 10 minutes using slow speed.

5.2.3.4 Filter the extract through a Whatman 2V filter paper into a 16-oz. bottle (Boston round, narrow mouth).

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5.2.3.5 Transfer a 55-ml aliquot (5-g equivalent) to a 250-ml separatory funnel and partition with hexane, Step 5.3.

5.2.4 Extraction of Muscle, Liver and Kidney Samples

5.2.4.1 Weigh a 25-g representative subsample into a blender jar. Add 250 ml of 20% water/acetonitrile. Blend the sample for 10 minutes using slow speed (use a variable transformer to regulate the speed).

5.2.4.2 Filter the extract through a Whatman 2V filter paper into a 16-oz. bottle (Boston round, narrow mouth).

5.2.4.3 Transfer a 54-ml aliquot (5-g equivalent) into a 250-ml separatory funnel and partition with hexane, Step 5.3.

5.3 Acetonitrile - Hexane Partition Step

5.3.1 Add 50 ml of hexane to the separatory funnel containing the sample in 20% water/acetonitrile from Step 5.2.1.4 (milk), 5.2.3.5 (eggs), 5.2.4.3 (muscle, liver and kidney samples).

5.3.2 Stopper and shake the funnel for 30 seconds, allow the two phases to separate, then drain the aqueous acetonitrile phase (bottom) into a 250-ml erlenmeyer flask. Transfer the hexane phase (top) to another 250-ml separatory funnel.

5.3.3 Repeat partitioning of the aqueous acetonitrile phase using 50 ml of fresh hexane. Drain the aqueous acetonitrile phase into a 500-ml round bottom flask.

5.3.4 Combine the two hexane phases and back partition using 50 ml of acetonitrile.

5.3.5 Drain the acetonitrile phase and combine with the aqueous acetonitrile phase in the 500-ml round bottom flask. Discard the hexane phase.

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5.3.6 Concentrate the combined acetonitrile phases to a small volume (approx. 5-10 ml) using a rotary evaporator. Proceed to the phosphoric-acid reflux step (Step 5.4.)

5.4 Phosphoric Acid Reflux

5.4.1 Add 100 ml of 85% phosphoric acid, approximately 1 g of cobalt chloride hexahydrate and two or three boiling chips to the flask containing the sample. (Step 5.2.2.6 or 5.3.6).

5.4.2 Place the flask in a 500-ml heating mantle (Note: Do not attach the reflux condenser at this time.) Heat the solution to boiling and monitor the boiling point of the solution using a 0-360°C thermometer, 2-in. immersion, until the temperature reaches $170^{\circ} \pm 2^{\circ}\text{C}$.

5.4.3 Remove the thermometer, attach the reflux condenser to the flask and reflux overnight (16 hours).

5.5 Basification

5.5.1 Allow the solution to cool for about 30 minutes, and add 50 ml of distilled water through the top of the condenser using an 8.5-cm funnel.

5.5.2 Add 200 ml of 25% sodium hydroxide solution in small increments through the top of the condenser. (Note: Heat is generated by addition of base to the acid solution. A recommended procedure is to add 10 ml of the base to the flask, swirl and let stand for five minutes, then add 20 ml, 30 ml, 40 ml, 50 ml and 50 ml increments of the base, allowing five to ten minutes between each addition and swirling the flask to facilitate mixing). Rinse the condenser with 25 ml of water.

NOTE: Use of gloves during the addition of the base to the flask is recommended for the safe handling of the base.

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5.6 Steam Distillation

- 5.6.1 Place the flask containing the basified extract in a 500-ml heating mantle after adding a few boiling chips and attach to the steam-distillation condenser apparatus shown in Figure 3.
- 5.6.2 Add 15 ml of hexane through the top of the steam distillation condenser.
- 5.6.3 Heat the flask to reflux and continue refluxing for one hour. The total heating time is about 1 1/4 hours.

5.7 Derivatization Step

- 5.7.1 Withdraw the hexane from the steam distillation apparatus using the solvent withdrawal tube in the side of the apparatus into a 25-ml graduated cylinder.

NOTE: The derivatization of 2,6-dimethylaniline should be done immediately after Step 5.6.3 to prevent losses. The hexane solution may be stored in a freezer if the derivatization must be done at a subsequent time.

- 5.7.2 Filter the hexane phase (top layer) through an 8.5-cm funnel containing absorbent cotton into a 125-ml separatory funnel. Use a pasteur pipet for this transfer. Alternatively, if the sample was kept in the freezer, the aqueous phase will be frozen and the top hexane layer is easily poured into the funnel containing absorbent cotton instead of using a pasteur pipet.
- 5.7.3 Rinse the absorbent cotton and funnel with 15 ml of dichloromethane into the separatory funnel to give a 1:1 mixture of hexane:dichloromethane.

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5.7.4 Add 100 μ l of trichloroacetyl chloride to the hexane:dichloromethane solution in the separatory funnel using an automatic pipette and let the solution stand at room temperature for 15 minutes. (Caution: Trichloroacetyl chloride is toxic and corrosive. Hence the addition should be done inside a hood. Also, do not leave the reagent bottle open for an extended period because the reagent is moisture sensitive).

5.7.5 Add 25 ml of 5% sodium bicarbonate solution and shake. Draw off the aqueous layer (bottom) and discard. Repeat the partition with an additional 25 ml of sodium bicarbonate solution.

5.7.6 Draw off the organic phase (top) into a 125-ml round bottom flask using a funnel containing an absorbent cotton plug. Rinse the funnel using 25 ml of dichloromethane.

5.7.7 Evaporate the sample to dryness using a rotary evaporator. (Note: Do not exceed a bath temperature of 30°C. Losses will be encountered if the bath temperature is higher because of the volatility of the derivative).

5.8 Alumina Column Cleanup

5.8.1 Fill a chromatographic column (19 mm i.d. containing a glass wool plug at the bottom) with hexane. Measure 30 ml of Grade V alumina using a graduated cylinder and add to the column. Gently tap to remove any trapped air bubbles. (The column is approximately 11 cm in height.)

5.8.2 Drain off the hexane until the liquid layer reaches the top of the alumina.

5.8.3 Load the sample (Step 5.7.7) onto the column using three 5-ml portions of hexane. Collect the eluate in a 250-ml round bottom flask.

5.8.4 Add 150 ml of hexane to the column and collect the eluate in the same flask.

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- 5.8.5 Evaporate the hexane eluate to dryness using a rotary evaporator (Note: Do not exceed a bath temperature of 30°C. Losses will be encountered if the bath temperature is higher because of the volatility of the derivative).
- 5.8.6 Add 5 ml of hexane to the flask to dissolve the sample. Pipette a 4-ml aliquot of the sample into a 20-ml sample vial. (Note: An N-Evaporator is not recommended for evaporation because of losses of the derivative encountered by using a stream of air).
- or
- 5.8.7 Transfer the sample after the alumina column clean up to a 20-ml concentration tube using approximately 10 ml of hexane.
- 5.8.8 Evaporate the sample to 1.0 ml or 2.0 ml using a rotary evaporator (bath temperature 30°C). The sample is now ready for GC analysis.

5.9 Silica Gel Column Cleanup (for Liver and Kidney Samples)

- 5.9.1 Fill a chromatographic column (19 mm i.d. containing a glass wool plug at the bottom) with hexane. Measure 30 ml of silica gel using a graduated cylinder and add to the column. Gently tap to remove any trapped air bubbles. (Alternatively, prepare the column using a slurry of silica gel in hexane until the column is approximately 4 inches in height).
- 5.9.2 Drain off the hexane until the liquid layer reaches the top of the alumina.
- 5.9.3 Load the sample (Step 5.8.5) onto the column using three 5-ml portions of hexane. Add an additional 50 ml hexane to the column. Discard the eluate.
- 5.9.4 Add 100 ml of 5% ethyl ether/hexane to the column and discard the eluate.
- 5.9.5 Add 150 ml of 10% ethyl ether/hexane to the column. Collect the eluate in a 250-ml round bottom flask.

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5.9.6 Evaporate the sample to dryness using a rotary evaporator. (Bath temperature 30°C).

5.9.7 Add 5 ml of hexane to the flask to dissolve the sample. Pipette a 4-ml aliquot of the sample into a 20-ml sample vial. (Alternatively, concentrate the sample to 2 ml using the concentration tube as in Steps 5.8.7 and 5.8.8). The sample is now ready for GC analysis.

6.0 GAS CHROMATOGRAPHIC ANALYSIS

The sample in step 5.8.6, 5.8.8 or 5.9.7 is analyzed by gas chromatography using an alkali flame ionization detector operated in the nitrogen-specific mode. The gas chromatographic conditions are given in Table I.

Alternatively, samples are analyzed by gas chromatography-mass spectrometry in the chemical ionization mode by selected ion monitoring (SIM) of the M+1 ion at m/e 268. The gas chromatography mass chromatography (GC-MS) conditions for the analysis are given in Table II.

6.1 Standardization

6.1.1 Prepare a stock solution containing 100 mg of N-(2,6-dimethylphenyl)-2,2,2-trichloroacetamide (TCA-DMA) in 100 ml of acetone. Serial dilutions should be made with hexane until a working solution containing 0.5 ng/ μ l is achieved.

6.1.2 Dilute the working solution (0.5 ng/ μ l) to yield standard solutions of 0.2 ng/ μ l, 0.1 ng/ μ l and 0.05 ng/ μ l. Standardize the gas chromatograph, operating under the conditions specified in Table I or II by injecting 4- to 8- μ l aliquots of the diluted solutions. This represents a working range of 0.2 ng to 4 ng for the derivative.

6.1.3 Determine the peak height or area for the injected standards. Typical chromatograms and standardization data of TCA-DMA standards using the GC-AFID detector are shown in Figure 4 and Table III, respectively. Typical chromatograms and standardization data of TCA-DMA standards using GC-MS are shown in Figure 5 and Table IV, respectively.

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6.1.4 Construct a standard curve, plotting detector response (peak height or area) versus nanograms injected. Typical standard curve for GC-AFID and GC-MS are presented in Figures 6 and 7, respectively. Alternatively, enter the standardization data into an appropriate electronic calculator (e.g., Texas Instrument Model TI55) to calculate a least square standard curve.

6.2 Detection of Sample Residues

6.2.1 Inject a 4-8 μ l aliquot of the sample in Step 5.8.6 or 5.8.8 into the gas chromatograph. Make appropriate dilutions of the sample to have the sample peak height within the range of the standard curve. Compare peak heights or areas of unknown samples with the standard curve or enter into the least square program of the calculator to determine the amounts of the derivative in the aliquot injected.

Typical chromatograms of tenderloin and omental fat samples using GC-AFID are shown in Figures 8 and 9, respectively. Typical chromatograms of milk and liver samples, using GC-MS are shown in Figures 10 and 11, respectively.

6.2.2 Calculate residue results as ppm equivalents of metalaxyl using the following equation:

$$\text{PPM} = \frac{\text{Amount TCA-DMA found (ng)}}{\text{mg crop injected}} \times 1.053 + R$$

where R is the recovery factor determined using a fortified control sample carried through the procedure and is expressed as a decimal (100% = 1.0, etc.).

The factor 1.053 is used to convert residues of TCA-DMA found into metalaxyl equivalents.

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7.0 DISCUSSION

- 7.1 Milk samples were screened at the 0.01 ppm level using the gas chromatography - mass spectrometry detection system. The recoveries for milk samples fortified at 0.01 to 0.10 ppm ranged from 52 to 76% with an average of $66 \pm 8\%$ ($n = 10$).
- 7.2 An interference peak was seen in selected samples of liver and kidney samples in the gas chromatographic analysis using the AFID detector. The samples analyzed by gas chromatography - mass spectrometry showed <0.10 ppm interference. Typical recoveries of liver samples fortified at 0.1 to 0.4 ppm ranged from 54 to 116% with an average of $83 \pm 23\%$ ($n = 5$).

8.0 REFERENCES

1. AG-330: "The determination of CGA-48988 and its metabolites in tobacco as 2,6-dimethylaniline using phosphoric acid reflux."
2. G. D. Veith and L. M. Kiwus, "An Exhaustive Steam-Distillation and Solvent-Extraction Unit for Pesticides and Industrial Chemicals," Bulletin of Environmental Contamination and Toxicology, Vol. 17, 1977, p. 631-636.

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TABLE I. GAS CHROMATOGRAPHIC CONDITIONS FOR GC-AFID ANALYSIS

| | |
|--------------------------------|---|
| <u>Instrument</u> | Tracor 200 equipped with an alkali flame ionization detector (Perkin-Elmer) |
| <u>Column Packing</u> | 3% Dexsil 300 on Gas Chrom Q (80/100 mesh) |
| <u>Column</u> | Pyrex 4' x 4 mm i.d. |
| <u>Temperatures</u> | |
| Column | 155°C |
| Injector | 265°C |
| Detector | 255°C |
| <u>Gas Flows</u> | |
| He carrier | 60 ml/min. |
| H ₂ reaction gas | 3.0 ml/min. (regulated) |
| Compressed air | 100 ml/min. |
| <u>Attenuation</u> | 1 x 8 |
| <u>Bead Current Setting</u> | 745 |
| <u>Minimum Detection Limit</u> | 0.5 nanogram |
| <u>Volumes Injected</u> | 4-8 µl |
| <u>Chart Speed</u> | 1 cm/minute |
| <u>Retention Time</u> | 3.7 minutes |

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TABLE II: INSTRUMENT CONDITIONS FOR GC-MS ANALYSIS

| | |
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| <u>Instrument</u> | Finnigan Model 3200 gas chromatograph - Mass Spectrometry operating in the chemical ionization mode with methane as the reactant and carrier gas. (Between the column and the MS interface, a "T" equipped with a toggle valve to a vacuum line is used to vent large volumes of solvent without impairing the functions of the mass spectrometer). |
| <u>Carrier gas:</u> | Methane flow adjusted to give 1,000 μ pressure in the ion source. |
| <u>Temperatures</u> | |
| Column | 165°C |
| Inlet | 230°C |
| Interface | 250°C |
| Transfer | 250°C |
| MS Manifold | 90°C |
| <u>MS Settings</u> | |
| Electron energy | 120 eV |
| Emission | 0.50 ma |
| Ion energy | 15V (volts) |
| Collector | 30 (volts) |
| Lens | 40 (volts) |
| Extractor | 10V (volts) |
| Electron multiplier | 2.0 (KV) |
| Mass range | 268.0 \pm 0.2 amu (scan range 0.2 amu) |
| Sensitivity | 10 ⁻⁸ |
| Ion current | |
| integrator | 100 |
| Recorder | 1.0 V |
| Chart speed | 1 cm/sec. |
| Vent time | 1.0 min. |
| Retention time | 3.5 min. |
| Minimum detection limit | 0.2 ng |

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TABLE III: TYPICAL STANDARD CURVE FOR GC-AFID ANALYSIS

| <u>Amount TCA-DMA Injected (Nanograms)</u> | <u>Peak Height (cm)</u> |
|--|---------------------------------|
| 1.0 | 1.9 |
| 8.0 | 15.0 |
| 2.0 | 2.9 |
| 4.0 | 6.8 |
| 0.2 | 0.3 |
| 1.0 | 1.4 |
| 2.0 | 3.1 |
| 4.0 | 6.8 |
| 8.0 | 15.3 |

See Figure 4 for plot.

Regression Data for the standard curve

n = 9 Correlation coefficient 0.9981 slope: 1.910
Intercept: -0.4991

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TABLE IV. TYPICAL STANDARD CURIVE FOR GC-MS ANALYSIS

| Amount TCA-DMA Injected (Nanograms) | Peak Height (cm) |
|--|------------------------|
| 2.0 | 12.9 |
| 0.25 | 2.0 |
| 0.5 | 3.9 |
| 1.0 | 7.0 |
| 0.5 | 3.5 |
| 1.5 | 10.2 |
| 0.25 | 1.8 |
| 0.5 | 3.2 |

See Figure 5 for plot.

Regression data for the standard curve

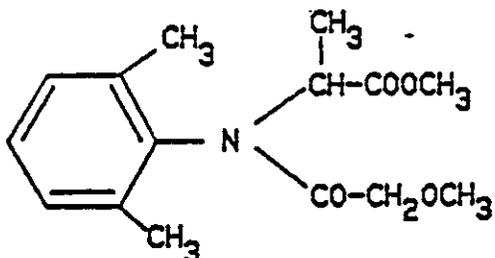
n=8 Correlation coefficient: 0.9980 Slope: 6.398

Intercept: 0.3643

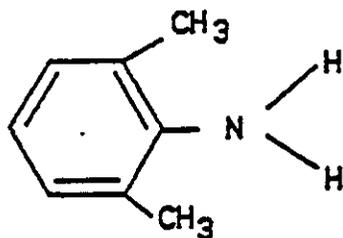
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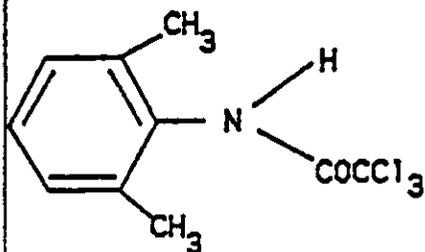
| | | |
|-------------------------------------|----------------------|---|
| PAGE 18 of 29 | METHOD No. AG-349 | SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN ANIMAL TISSUES, MILK AND EGGS AS 2,6- DIMETHYLANILINE |
| EDITION | | |
| SUBMITTED BY: K. Balasubramanian | | APPROVED BY: |



Metalaxyl, CGR-48988
N-(2,6-Dimethylphenyl)-N-
(methoxyacetyl)-alanine
methyl ester
 $C_{15}H_{21}O_4N$



CGR-72649
2,6-dimethylaniline



TCA-DMA
N-(2,6-Dimethylphenyl)-
2,2,2-trichloroacetamide

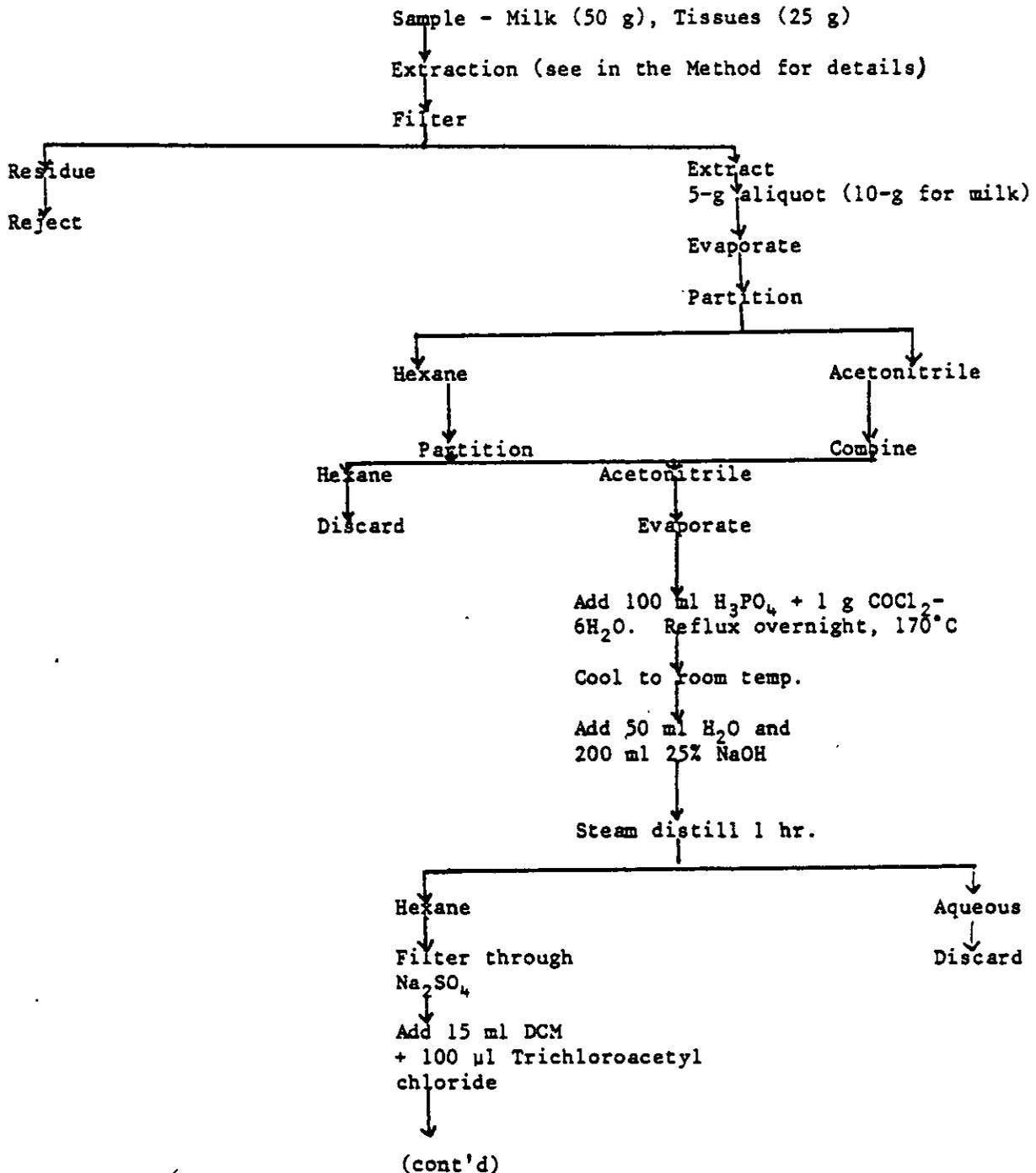
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Figure 1: CHEMICAL NAMES AND STRUCTURES

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|-------------------------------------|----------------------|---|
| PAGE 19 of 29 | METHOD No. AG-349 | SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN ANIMAL TISSUES, MILK AND EGGS AS 2,6- DIMETHYLANILINE |
| EDITION | | |
| SUBMITTED BY: K. Balasubramanian | | APPROVED BY: |

FIGURE 2: FLOW DIAGRAM OF THE ANALYTICAL PROCEDURE FOR THE DETERMINATION OF METALAXYL AND ITS METABOLITES IN MEAT AND MILK

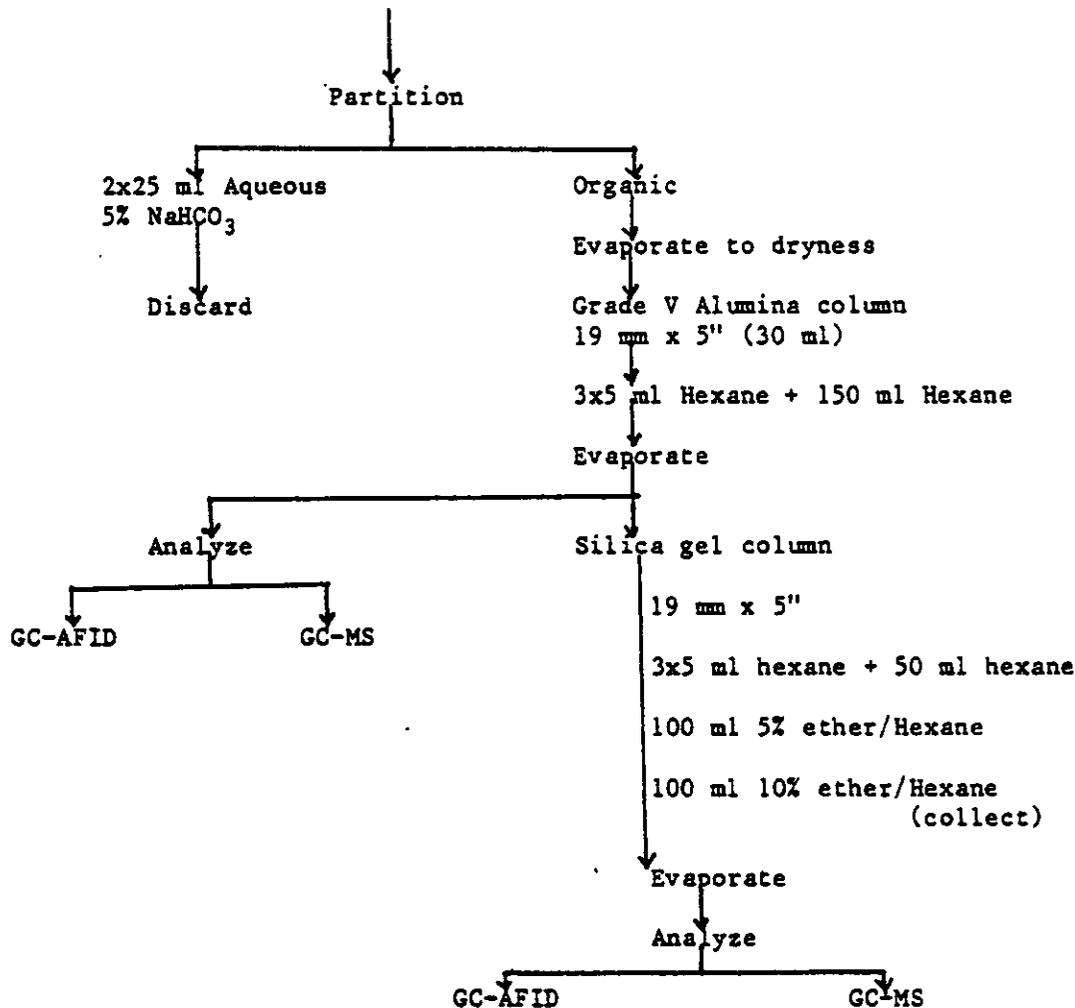


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| PAGE 20 of 29 | METHOD No. AG-349 | SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN ANIMAL TISSUES, MILK AND EGGS AS 2,6- DIMETHYLANILINE |
| EDITION | | |
| SUBMITTED BY: K. Balasubramanian | | |
| | | APPROVED BY: |

FIGURE 2: FLOW DIAGRAM OF THE ANALYTICAL PROCEDURE FOR THE DETERMINATION OF METALAXYL AND ITS METABOLITES IN MEAT AND MILK Continued)

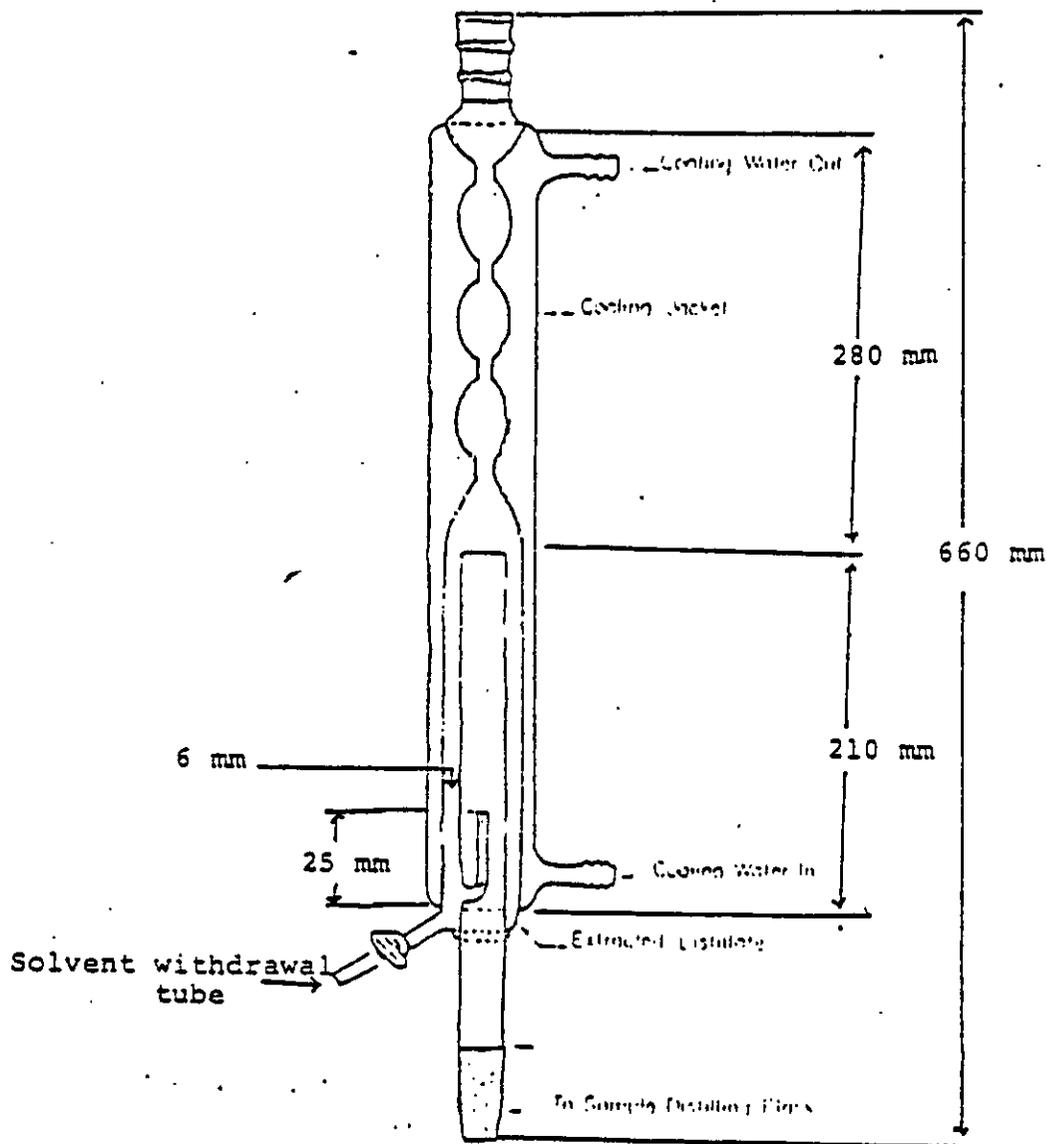


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|-------------------------------------|----------------------|---|
| PAGE 21 of 29 | METHOD No. AG-349 | SUBJECT ANALYTICAL METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF METALAXYL IN ANIMAL TISSUES, MILK AND EGGS AS 2,6- DIMETHYLANILINE |
| EDITION | | |
| SUBMITTED BY: K. Balasubramanian | | |
| | | APPROVED BY: |

FIGURE 3: EXHAUSTIVE STEAM-DISTILLATION AND SOLVENT-EXTRACTION APPARATUS



Note: The length of the tube (25 mm) and the space between the jackets (6 mm) are important. These dimensions assure that approximately 15 ml of organic solvent is contained in the well between the jackets.

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**FIGURE 4: TYPICAL GAS CHROMATOGRAPHY OF TCA-DMA STANDARDS
USING GC-AFID**

38 Dexsil
GC-AFID: Detector

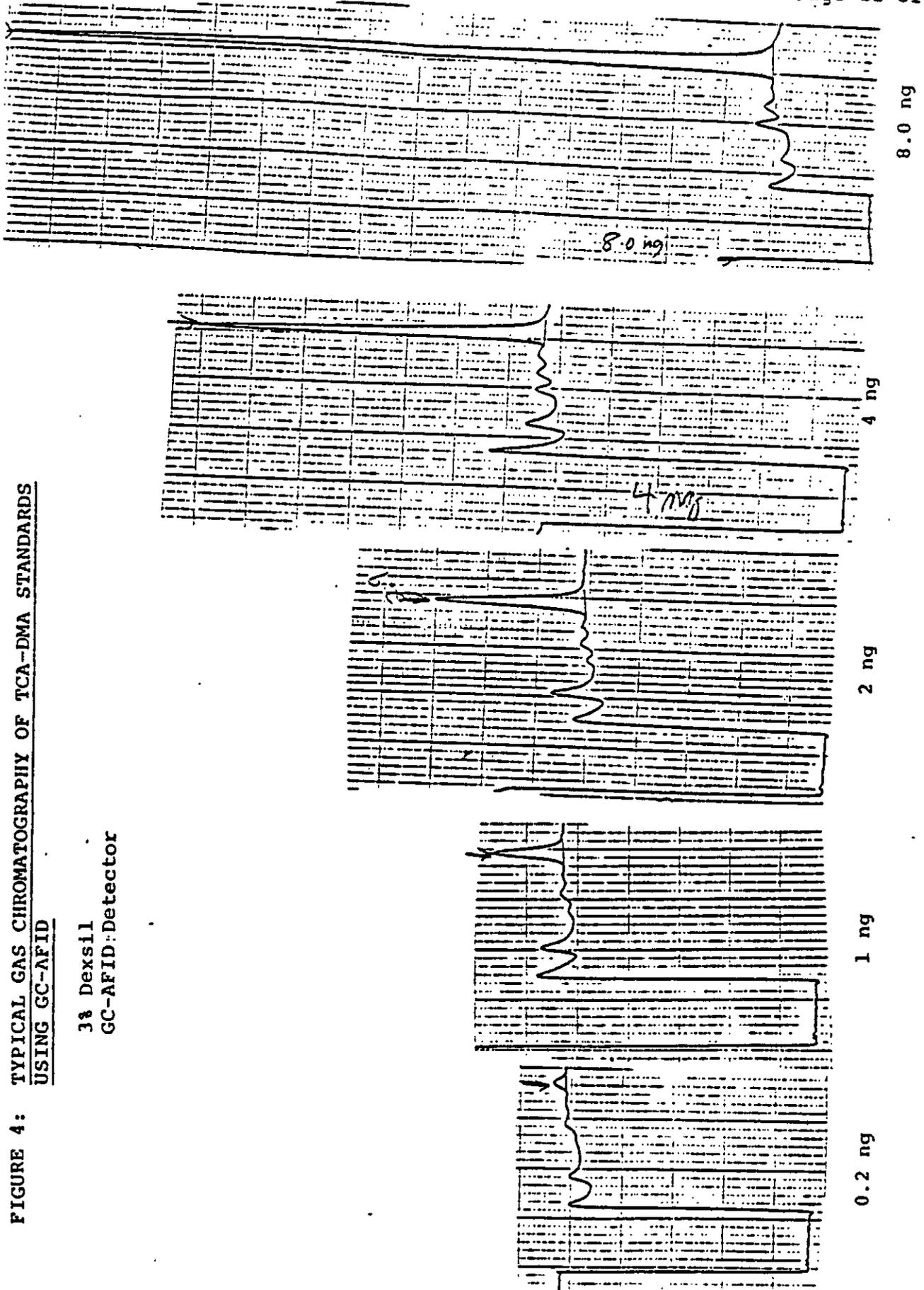
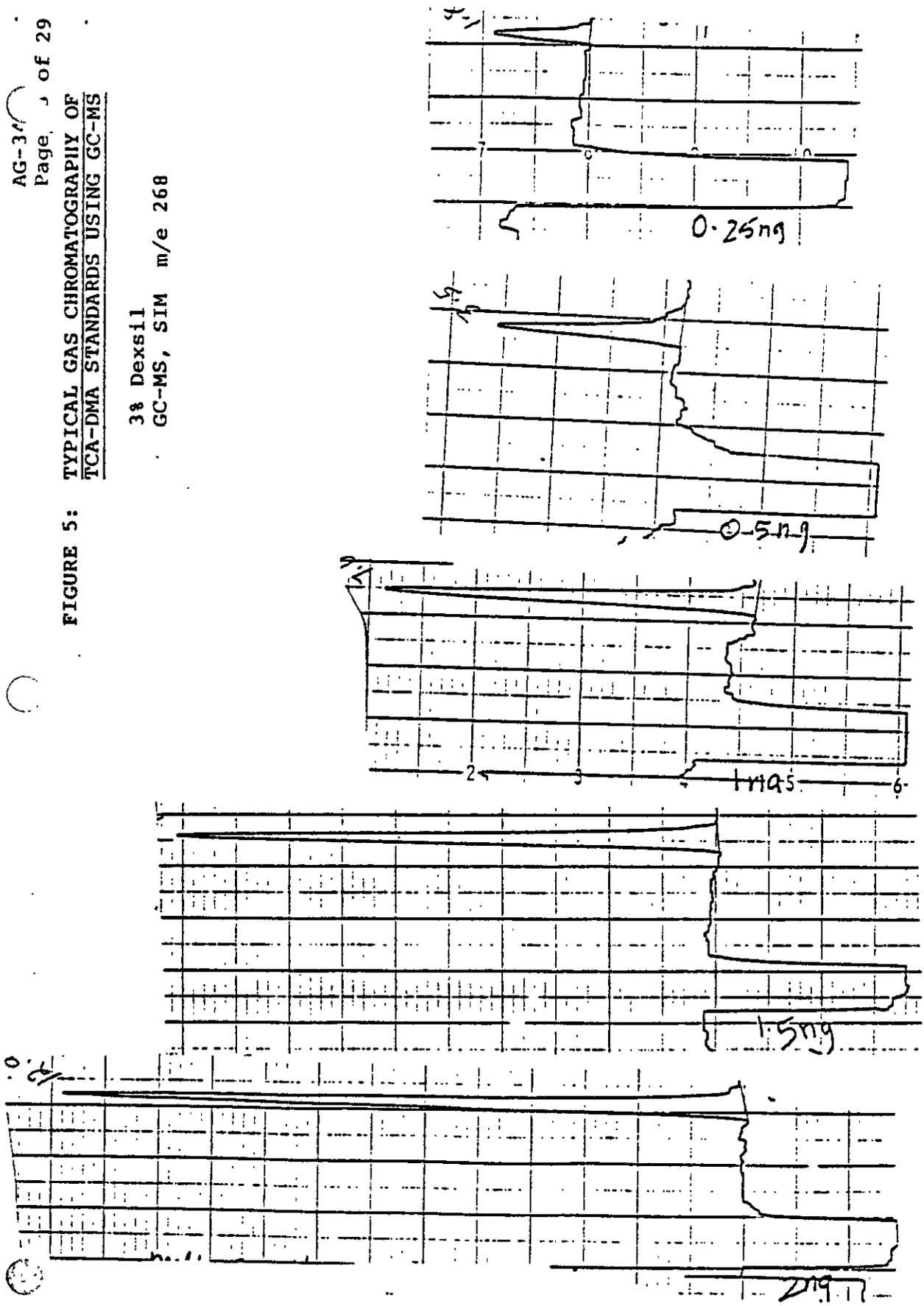


FIGURE 5: TYPICAL GAS CHROMATOGRAPHY OF TCA-DMA STANDARDS USING GC-MS

38 Dexsil
GC-MS, SIM m/e 268



2.0 ng
1.5 ng
1.0 ng
0.5 ng
0.25 ng

FIGURE 6: TYPICAL STANDARD CURVE OF TCA-DMA BY ALKALI FLAME IONIZATION DETECTOR (SEE TABLE III FOR DATA) 133

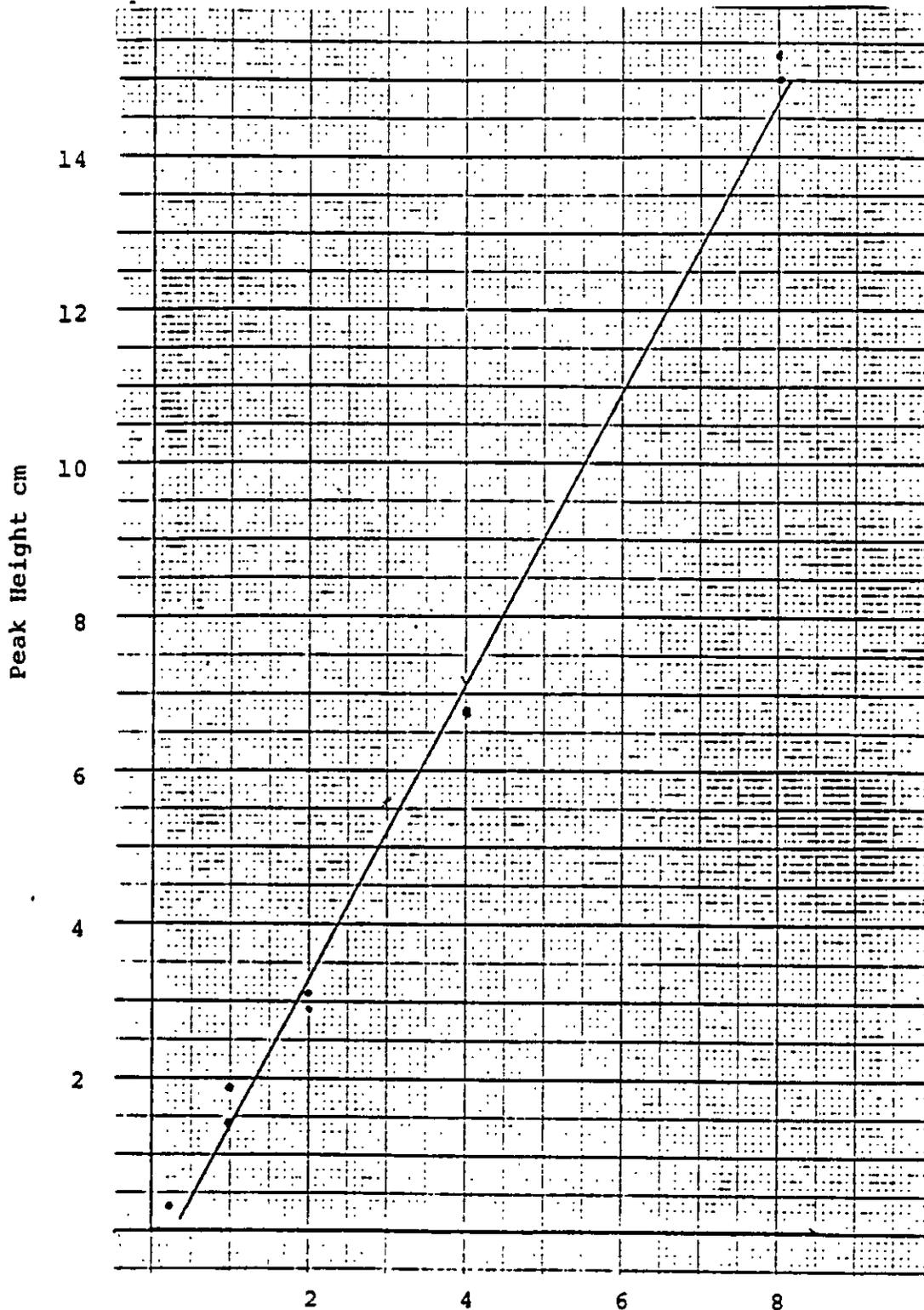


FIGURE 7: TYPICAL STANDARD CURVE OF TCA-DMA BY GAS CHROMATOGRAPHY - MASS SPECTROMETRY (SEE TABLE IV FOR DATA)

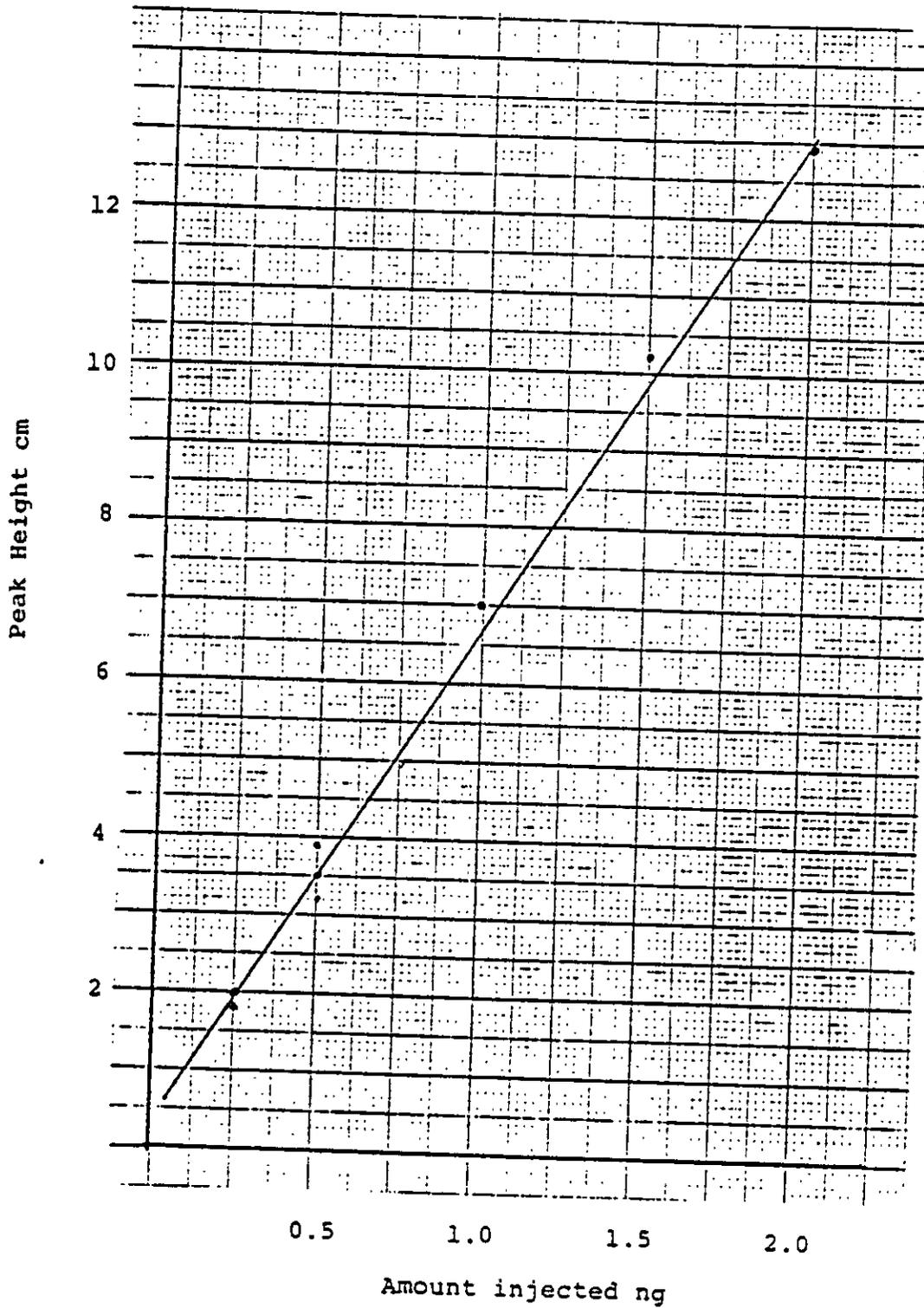
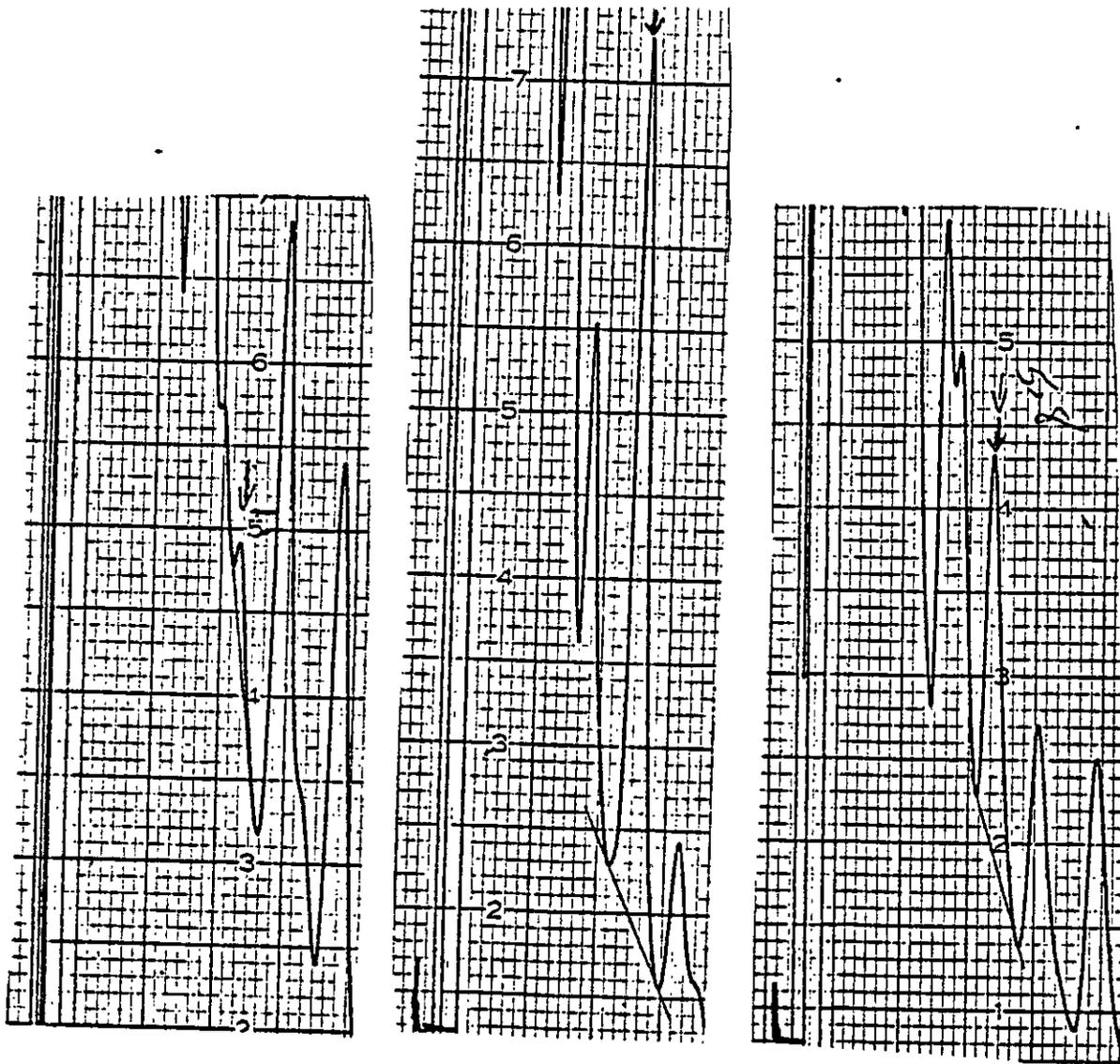


FIGURE 8: TYPICAL GC-AFID SCANS FOR TENDERLOIN SAMPLES

AG-A 5716



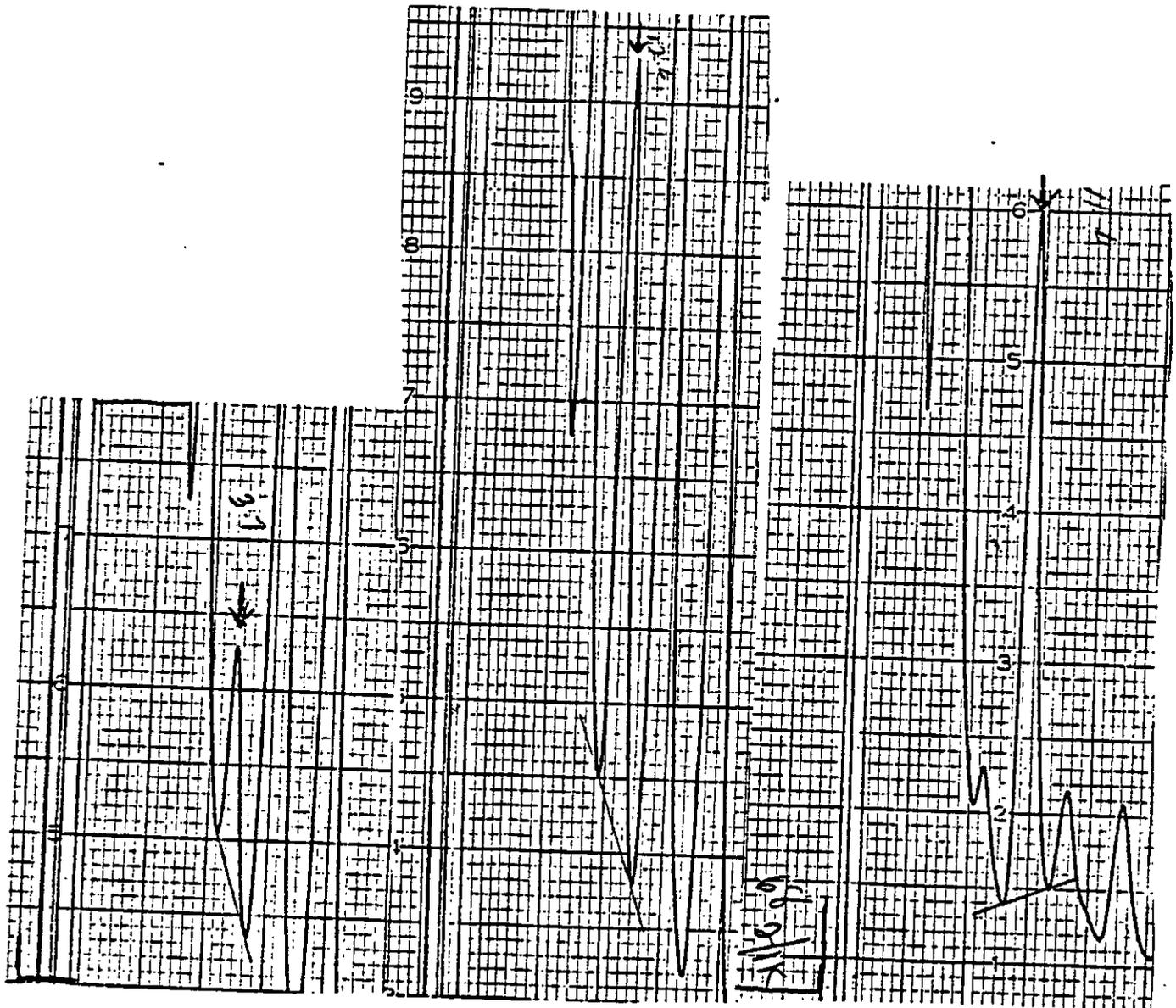
Check
 200 mg injected
 Found <1.0 ng
 <0.05 ppm

Check + 0.39 ppm
 metalaxyl
 30 mg injected
 Found 9.1 ng
 0.32 ppm
 83% recovery

Check + 0.2 ppm
 metalaxyl
 40 mg injected
 Found 4.63 ng
 0.122 ppm
 61% recovery

FIGURE 9: TYPICAL GC-AFID SCANS FOR OMENTAL FAT SAMPLES

AG-A 5716



Check
200 mg injected
Found 2.5 ng
<0.05 ppm

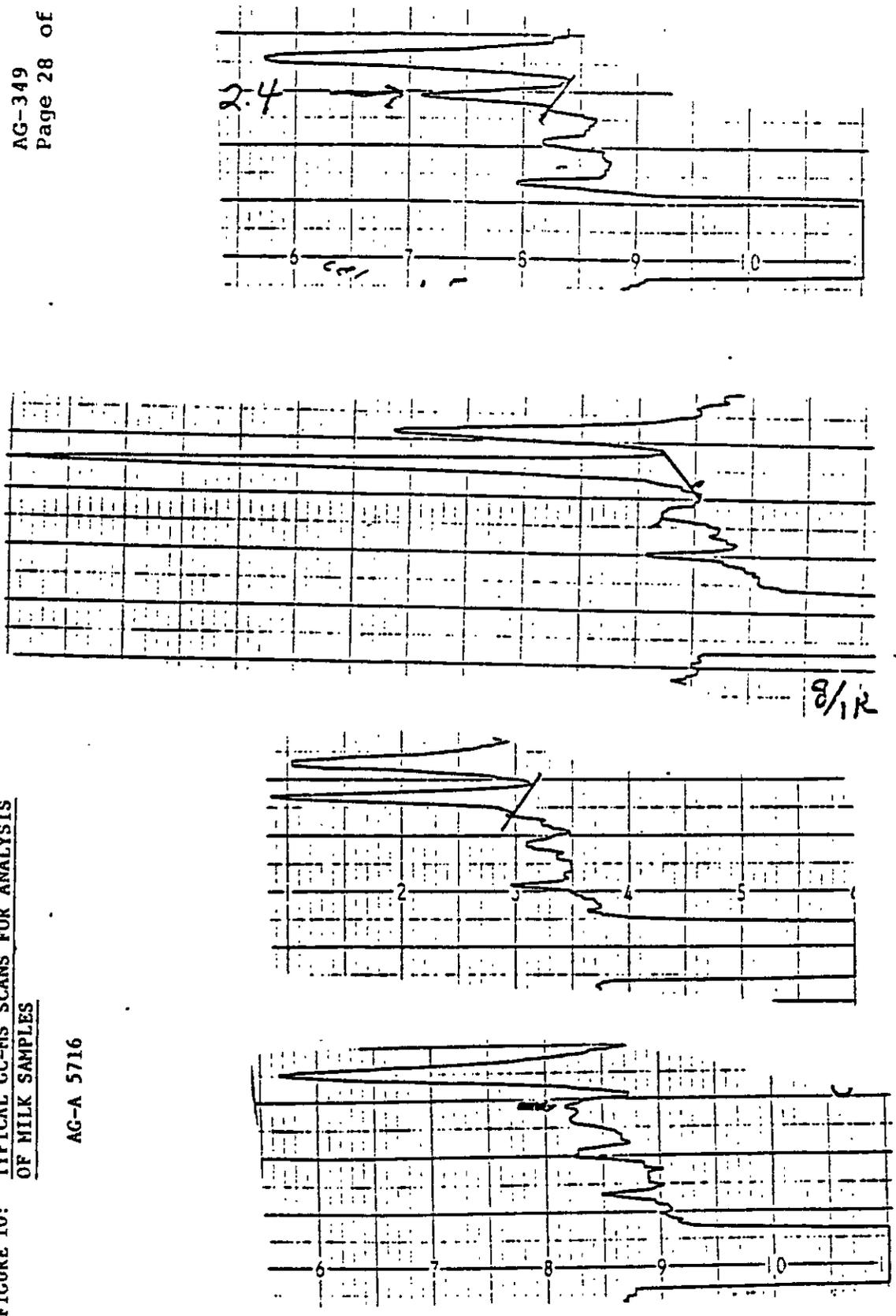
Check + 0.05 ppm
metalaxyl
200 mg injected
Found 8.3 ng
0.031 ppm
62% recovery

Check + 0.65 ppm
metalaxyl
20 mg injected
Found 7.5 ng
0.383 ppm
59% recovery

FIGURE 10: TYPICAL GC-MS SCANS FOR ANALYSIS OF MILK SAMPLES

AG-A 5716

AG-349
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Check
80 mg injected
Found <0.2 ng
<0.01 ppm

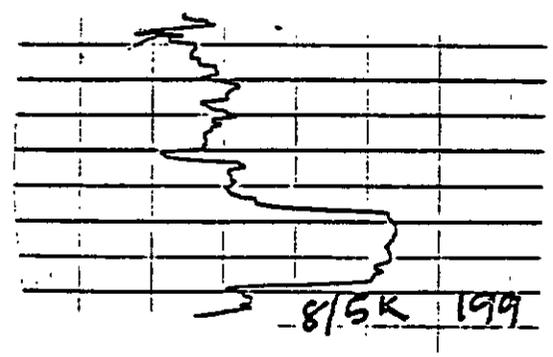
Check + 0.01 ppm
metalaxyl
80 mg injected
Found 0.48 ng
0.006 ppm
63% rec.

Check + 0.02 ppm
metalaxyl
80 mg injected
Found 1.13 ng
0.0147 ppm
74% rec.

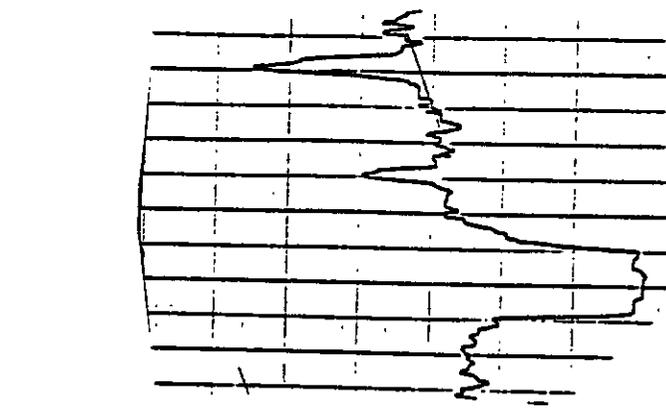
M-181 15 ppm treated
14 day
80 mg injected
Found 0.3 ng
<0.01 ppm

FIGURE 11: TYPICAL GC-MS SCAN FOR ANALYSIS OF LIVER SAMPLES

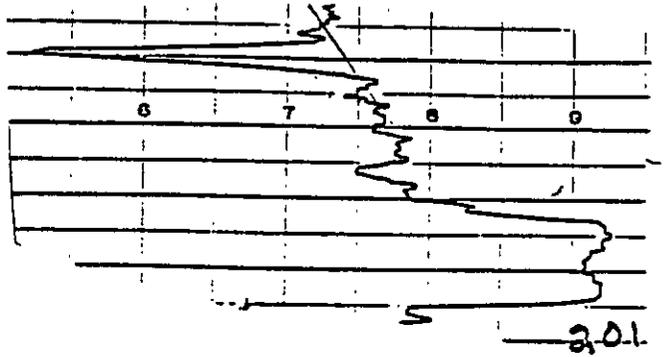
AG-A 5716



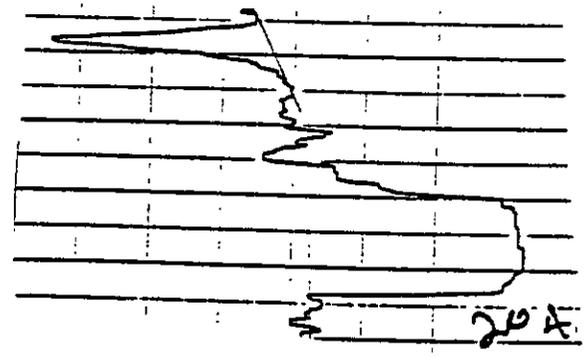
Check
8 mg injected
Found <0.5 ng
<0.1 ppm



Check + 0.1 ppm
metalaxyl
8 mg injected
Found 0.64 ng
85% rec.



Check + 0.2 ppm
metalaxyl
8 mg injected
Found 1.05 ng
69% rec.



1-5-A treated (15 ppm)
28 day
8 mg injected
Found 0.77 ng
0.13 ppm